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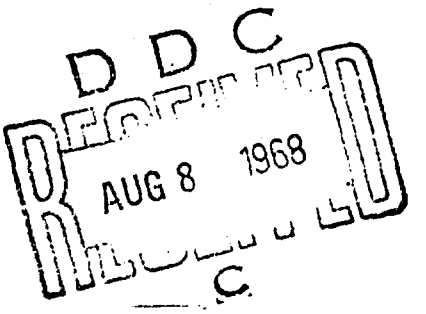

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FLUORESCENT ANTIBODIES IN THE DIAGNOSIS OF HERPATIC ILLNESSES

by

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The basis of this study is the problem of the keratitides where it is not possible to determine with certainty whether there is actually still a herpatic infection or only a trophic disturbance; there are also certainly cases of herpes virus uveititis where the diagnosis can only very rarely be ascertained by the discovery of viruses in the water of the eye, because the virus cannot circulate freely in the front space because they are bound to cells.

The herpes simplex virus, although it is one of the large viruses, cannot be seen with the microscope; in humans it does not built typical enclosing bodies, but it does in the cornea of rabbits after experimental infection. Therefore the diagnosis has to be obtained from the clinical picture, which does hardly ever deceive us in fresh cases of keratitis; but in cases of deep keratitis or uneititis we often have to rely completely on suspicions.

The method of the fluorescent marked antibodies according to A. H. Coons, with which the proof of intraoccular antibody production was already made (Witmer), was already used by Vazzo and Pauluzzi in the experimental herpes infection of rabbits. They use however, a somewhat complicated socalled "sandwich technique" after Weller and Coons, with fresh serum of herpes convalescents and fluorescin marked antihuman gamma-globin.

We have chosen the direct method, as described a short time ago by H. Kaufmann for the diagnosis of herpatic keratitides of humans. Sea horses were actively immunized during several months with the virus-containing fluid obtained from fresh tissue cultures. The antisera of

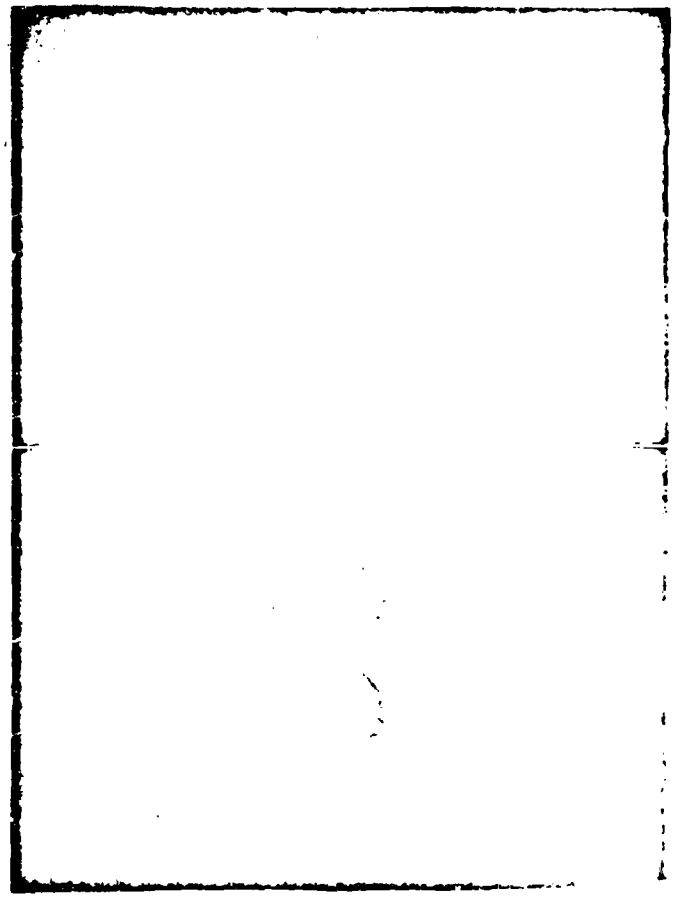
these animals with throughout high titers were then conjugated with fluorescin isothiocyanate. This dye has the property of binding itself strongly to protein molecules, and therefore also to antibodies, without changing their immunological properties. Under activation with ultraviolet light the antibodies which are dyes in this way fluoresce strongly and can easily be located in a fluorescent microscope. We have tried to eliminate non specific fluorescence in our experiments by adsorbing the marked antiserum with a fresh tissue culture of epithelial cancer cells.

In cell cultures of epithelial human cancer cells which are inocculated with herpes virus there is after 24 to 48 hours an already macroscopically visible cytopathogenic effect: the otherwise very symmetrical sheets of cells become irregular and often have gaps. Microscopically the normal cells, which are not infected by virus, usually have an elongated shape with a clear nucleus and often long extensions. They cannot be colored, but they have a slight proper fluorescence. The cells which are infected with virus however swell up, become grainy and absorb plenty of fluorescin antibodies. Often there is an extremely strong fluorescing zone in the near surrounding of the nucleus. The cells appear very bright, and in advanced stages often no more clear structure can be seen.

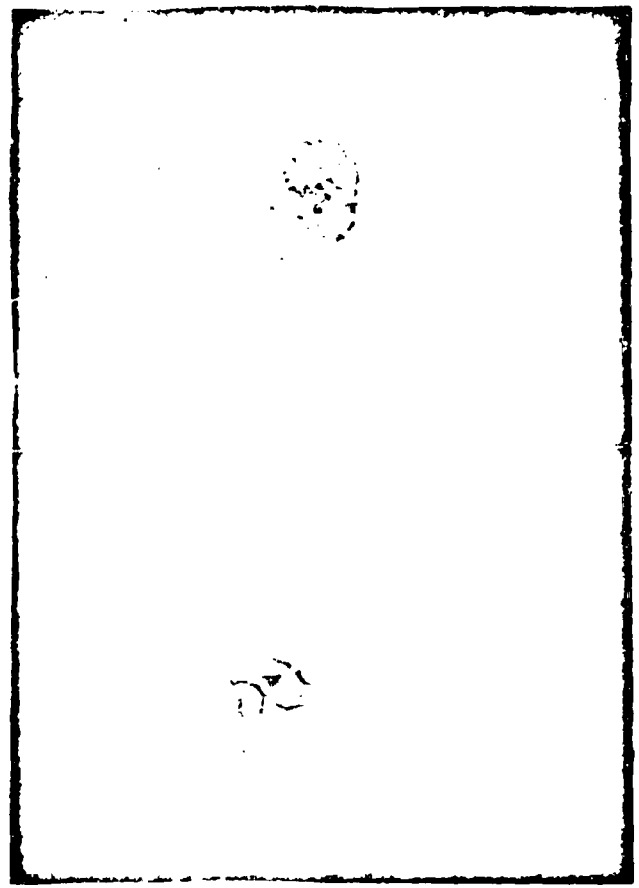
In corneal epithelia of fresh or also older herpetic keratitides the following picture is observed: besides weakly visible cells with slight proper fluorescence there are other cells which fluoresce strongly. These last cells must have absorbed marked antibodies, and therefore they must contain virus. Here also the impression is often gained that there is around the nucleus a zone of stronger fluorescence.

It is therefore possible with this method to prove the existence of herpes virus in the tissue culture and also in the corneal epithelia of clinical infections. Here we must certainly remark that in all cases which were investigated so far there was no doubt about the clinical diagnosis. So far no positive results were obtained with cells from the water of the eye, but only one questionable case of virus uveititis was specially investigated so far.

The method of the fluorescin marked antibodies is however not only suitable for finding viruses, but also just as well for bacterial or mycotic infections. Because of its great specificity this method is far preferable to the normal dying methods. It is not impossible that most germs, after they have been phagocyted by the macrophages of the water of the eye or the tissue cells of the conjunctiva have changed so much morphologically that they fail to give a typical microscopic picture. But such ruined germs still contain antigens, which can react with a specific marked antibody.



Figures 1 and 2. Cytopathogenic effect in cancer cell cultures. Fluorescence of virus containing cells.



Figures 3 and 4. Corneal epithelia in cases of herpatic keratitis. Cells with weak proper fluorescence and strongly fluorescent virus containing cells.

We believe therefore that this method will bring new interest, not only in the cytology of the conjunctiva and the cornea, (for example in the diagnosis of trachoma or epidemic kerato-conjunctivitus), but also in the cytology of the water of the eye, and that this method can open further advances in the diagnosis of the various illnesses.

In this place I must express my sincere gratitude to Dr. Wulff from the Swiss serum and vaccine institute in Bern for her valuable cooperation.

Literature

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Discussion

R. Rintelen (Basel): Mr. Witmer must be congratulated for his very promising start on the way to virological investigation of the water of the eye. Our organization was clearly justified in electing him as a delegate to the international organization against trachoma.

P. Verrey (Zurich): I want to congratulate my colleague Mr. Witmer with his result. We are at the moment in the phase where data have to be collected which can only later be used for diagnosis. From my own experience I know that it is not always easy to come out of that stage. I hope that in a few years this method will be widely used.